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# OPTIMUM TEMPERATURE AND RELATIVE HUMIDITY FOR SPORE GERMINATION AND GERM TUBE GROWTH OF *CURVULARIA PALLESCENS* ON GLASS SLIDES AND MAIZE LEAF

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ABSTRACT. — Germination of spores and growth of ensuing germ tubes of *Curvularia pallescens*, were investigated on glass slides and on excised maize leaves. Investigations were carried out under temperatures of 15, 20, 25, 30, 35 and 40°C and relative humidity values of 70, 75, 80, 85, 90, 95 and 100 %. The results showed that optimum conditions of temperature and relative humidity for spore germination and germ tube growth of the fungus on both glass slides and excised maize leaves were 25 to 30°C and 95 to 100 % RH respectively. However, germination was greater on maize leaves than on glass slides.

RÉSUMÉ. – La germination des spores et la croissance des tubes germinatifs de *Curvularia pallescens* sont examinées sur lames de verre et sur feuilles de maïs excisées. Les examens sont réalisés à l'air libre, à des températures de 15, 20, 25, 30, 35 et 40°C, et à des humidités relatives de 70, 75, 80, 85, 90, 95 et 100 %. Les résultats montrent que les conditions optimales de température et d'humidité relative, pour la germination de la spore et la croissance du tube germinatif du champignon, sur lames de verre et sur feuilles de maïs excisées, sont respectivement de 25 à 30°C et de 95 à 100 %. Toutefois, on obtient une meilleure germination sur feuilles de maïs que sur lames de verre.

KEY WORDS: *Curvularia pallescens*, spore germination, germ tube growth.

## INTRODUCTION

*Curvularia* leaf spot is a disease of maize and has in the last twenty years been reported to occur in most maize growing areas of the world (MABADEJE, 1969). In most studies of this disease, emphasis has been given on symptomatology and identification of the causal agent, *Curvularia pallescens* Boed. (FAJEMISIN, 1975; MABADEJE, 1969). However, environmental factors affecting spore germination and germ tube growth have not been investigated in detail

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even though such informations could aid to understand the physiology and its relationship to its host.

In this paper, we sought to determine temperature and relative humidity conditions required for optimum germination and germ tube growth of *C. pallescens* either on the surfaces of glass slides or maize leaves.

## MATERIALS AND METHODS

The isolates of *Curvularia pallescens* used during this study was obtained from diseased maize leaves at the National Cereals Research Institute, Moor Plantation, Ibadan, Nigeria. It was maintained in culture by repeated transfers on potato dextrose agar in standard petri-dishes.

Whenever it was desired to use spore suspension, a small quantity (10 ml) of sterile distilled water was poured on to each of 12 days old sporulating cultures with the aid of a bent glass rod, the spores were dislodged and put into suspension in the water. Spore suspensions were filtered through a double layer of sterile fine muslin cloth to remove the hyphae. Filtrates were then poured into McCartney bottles and centrifuged at 2750 x g for five minutes. The supernatant was discarded and the spore suspension was concentrated by centrifuging as required with distilled water. Fusch's Rosenthal haemocytometer was used for the standardization of the spores suspension.

The relative humidity (RH) values used were 70, 75, 80, 85, 90, 95 and 100 %. They were obtained by preparing saturated solutions of various salts in accordance with the method of WINSTON & BATES (1960). The temperature used : 15, 20, 25, 30, 35, and 40°C, were obtained by adjusting the settings on a series of Gallenkamp incubators. For each desired RH value, three quarters of the basement parts of separate desiccators were filled with the appropriate salt solutions and a piece of wire gauze placed at the constricted region, few centimeter above the liquid. The desiccators were covered and opened only when specimens were put in or taken out. The edge of the desiccators lids were smeared with vaseline before closing in order to maintain the desired RH values and then placed inside the incubator for the desired temperature values.

Spore germination on glass slides, was determined by placing a drop (0.1 ml) of the spore suspension containing $1.0 \times 10^4$ spores/ml of sterile distilled water at the centre of a clean microscope slide. Slides were air dried for approximately ten minutes and the inoculated sites were inverted on the cavity of specimen glass cubes in the desiccators containing the appropriate RH salt solutions. Five replicates were made with all combinations of temperature and RH values.

Germination and germ tube growth were determined after 24 hours incubation. For germination counts, a total of 400 to 500 spores were examined at random per replicate and percentage germination was determined.

Spore germination on maize leaves was carried out on four 21 days old maize cultivar leaves. The cultivars were Msc-02, Milho, T.T. and Igbira. For each

cultivar, leaves were cut into 3 x 7 cm pieces and treated with a drop of the spore suspension similar to those on the glass slides. Spore germination was investigated on both upper and lower surfaces of the four maize cultivars used in the tests.

In one set of experiments, spore germination was carried out at a constant temperature of 25°C but at RH values of 95 and 100 %. In another, RH of 100 % but at temperature of 15, 20, 25, 30, 35 and 40°C.

Following incubation, the leaves were placed on glass slides, stained with lactophenol cotton blue and warmed on a hot plate for a few seconds to clear the leaves and stain the spores. Spores were then examined and the percentage germination determined.

## RESULTS

Spore germination on glass slides occurred at all temperatures except 40°C. Of the seven RH values used, germination occurred only at 95 and 100 %; consequently, only the results for 95 and 100 % RH are shown in Tables 1 and 2.

Table 1 – Effect of temperature and relative humidity on spore germination and germ tube growth of *Curvularia pallescens*, on glass slides.

Tableau 1 – Effet de la température et de l'humidité relative sur la germination des spores et sur la croissance des tubes germinatifs de *Curvularia pallescens*, sur lames de verre.

| Incubation Relative Humidity (%) | Temperature °C | | | | | | R.H. Mean |
|---|---|---|---|---|---|---|---|
| | 15 | 20 | 25 | 30 | 35 | 40 | |
| Germination (%) | | | | | | | |
| 95 | 28 | 53 | 88 | 89 | 40 | 4 | 49.8 b |
| 100 | 48 | 66 | 92 | 93 | 50 | 3 | 58.7 a |
| Temp. mean | 38.0 c | 59.5 b | 90.0 a | 91.0 a | 45.5 c | 3.5 d | |
| Intercalary germination (%) | | | | | | | |
| 95 | 31 | 50 | 61 | 60 | 40 | 0 | 40.3 b |
| 100 | 41 | 78 | 80 | 78 | 53 | 0 | 55.0 a |
| Temp. mean | 36.0 d | 64.0 b | 70.5 a | 69.0 a | 45.5 c | 0.0 c | |
| Germ tube branching (%) | | | | | | | |
| 95 | 41 | 60 | 72 | 61 | 36 | 1 | 45.2 b |
| 100 | 46 | 70 | 79 | 60 | 50 | 1 | 51.0 a |
| Temp. mean | 43.5 c | 65.0 b | 75.5 a | 60.5 b | 43.0 c | 1.0 d | |
| Length of germ tubes (µm) | | | | | | | |
| 95 | 200 | 281 | 362 | 408 | 316 | 31 | 281 b |
| 100 | 216 | 416 | 764 | 871 | 394 | 41 | 450 a |
| Temp. mean | 208 a | 348 c | 563 b | 639 a | 355 a | 36 e | |

Nota : – Mean values not followed by the same letter are significantly different (P = 0.05) according to Duncan's multiple range test.
– Data were obtained from a total of about 400 spores counted after 24 hours of incubation.

Table 2 – Variance analysis for the effect of temperature and relative humidity on spore germination and germ tube growth of *Curvularia pallescens*, on glass slides.

Tableau 2 – Analyses de variance pour l'effet de la température et de l'humidité relative sur la germination des spores et sur la croissance des tubes germinatifs de *Curvularia pallescens*, sur lames de verre.

| Source of variation | Degrees of freedom | Mean squares | | | |
|---|---|---|---|---|---|
| | | Germination | Intercalary germination | Germ tube branching | Length of germ tube |
| R.H. | 1 | 108.69 ** | 2 089.26 ** | 3 638.44 ** | 546 219.84 ** |
| Error (a) | 8 | 16.73 | 23.16 | 24.11 | 489.43 |
| Temperature | 4 | 4 951.65 ** | 9 297.88 ** | 17 522.19 ** | 1 340 066.50 ** |
| R.H. x Temp. | 4 | 628.00 ** | 3 634.80 ** | 5 821.62 ** | 442 330.45 ** |
| Error (b) | 32 | 48.91 | 51.31 | 62.31 | 890.64 |

* = significant at P=0.05

** = significant at P=0.01

Temperature and RH had considerable individual and combined effects on percentage germination, ability to germinate in more than one of the spore cells (intercalary germination), branching and length of the germ tubes (Tab. 1, 2).

Table 3 – Effect of relative humidity and maize cultivars (at 25°C) on spore germination and germ tube growth of *Curvularia pallescens*.

Tableau 3 – Effet de l'humidité relative et de différents cultivars de maïs (à 25°C) sur la germination des spores et sur la croissance des tubes germinatifs de *Curvularia pallescens*.

| Incubation Relative Humidity (%) | Maize cultivars | | | |
|---|---|---|---|---|
| | Msc-02 | Milho | Igbira | T.T. |
| Germination (%) | | | | |
| 95 | 89 b | 90 b | 91 b | 86 b |
| 100 | 97 a | 100 a | 99 a | 99 a |
| Intercalary germination (%) | | | | |
| 95 | 64 b | 65 b | 60 b | 61 b |
| 100 | 77 a | 80 a | 81 a | 79 a |
| Germ tube branching (%) | | | | |
| 95 | 80 b | 78 b | 77 b | 79 b |
| 100 | 91 a | 88 a | 89 a | 92 a |
| Length of germ tubes (µm) | | | | |
| 95 | 390 b | 403 b | 389 b | 420 b |
| 100 | 890 a | 916 a | 881 a | 989 a |

Nota : – For each trait and within each column, values not followed by the same letter are significantly different at P = 0.05 (Duncan's multiple range test).
– Data were obtained from a total of about 400 spores counted after 24 hours of incubation.

Relative humidity of 100 % was significantly better for spore germination than 95 % RH. Temperature of 25 and 30°C were optimum for germination and germ tube growth. However, when temperature regimes were considered along with RH, the optimum temperature was narrower with 95 % than 100 % (Tab. 1). Generally, temperature and RH regimes for germination were wider than those for germ tube growth.

In the case of maize leaves, germination and growth parameters were optimum at 100 % RH (Tab. 3). With these leaves, no significant effect of the cultivar on any of the spore germination traits measured could be observed (Tab. 3, 4). However, effects of RH and the cultivars x RH interaction were significant. For the germination and germ tube growth parameters, 100 % RH was optimum (Tab. 3).

Table 4 — Variance analysis for the effect of relative humidity and maize cultivars (at 25°C) on spore germination and germ tube growth of *Curvularia pallescens*.

Tableau 4 — Analyses de variance pour l'effet de l'humidité relative de différents cultivars de maïs (à 25°C) sur la germination des spores et sur la croissance des tubes germinatifs de *Curvularia pallescens*.

| Source of variation | Degrees of freedom | Mean squares | | | |
|---|---|---|---|---|---|
| | | Germination | Intercalary germination | Germ tube branching | Length of germ tube |
| Cultivars | 3 | 9.29 | 61.11 | 48.07 | 774.50 |
| Error (a) | 12 | 18.24 | 29.10 | 24.91 | 916.42 |
| R.H. | 1 | 145.00 * | 569.19 ** | 1 572.01 ** | 128 664.09 ** |
| Cult. x R.H. | 3 | 88.60 * | 123.41 * | 177.87 ** | 33 180.00 |
| Error (b) | 13 | 21.61 | 30.10 | 24.90 | 3 084.73 |

* = significant at P=0.05
** = significant at P=0.01

Temperature had a significant effect on spore germination and germ tube growth. But, the cultivar on which spores germinated had no effect on those phenomena (Tab. 5, 6). Temperature regimes of 25-30°C were the optimum for spore germination and germ tube growth whereas intercalary germination and germ tube branching had their optimum at 25°C.

Spore germination on glass slide was significantly different from those on maize leaves surfaces (Tab. 7). Furthermore, spore germination and growth of germ tubes on adaxial surfaces of maize leaves were not different from those on the abaxial surfaces, thus results were shown for one surface only.

Table 5 – Effect of temperature and maize cultivars (at 100 % R.H.) on spore germination and germ tube growth of *Curvularia pallescens*.

Tableau 5 – Effet de la température et de différents cultivars de maïs (à 100 % R.H.) sur la germination des spores et sur la croissance des tubes germinatifs de *Curvularia pallescens*.

| Temperature °C | Maize cultivars | | | |
|---|---|---|---|---|
| | Msc-02 | Milho | Igbira | T.T. |
| Germination (%) | | | | |
| 15 | 52 d | 50 d | 48 d | 55 d |
| 20 | 76 b | 80 b | 77 b | 82 b |
| 25 | 98 a | 99 d | 100 a | 96 a |
| 30 | 96 a | 95 a | 97 a | 97 a |
| 35 | 63 c | 63 c | 71 c | 72 c |
| Intercalary germination (%) | | | | |
| 15 | 42 d | 43 d | 52 c | 54 c |
| 20 | 71 b | 70 b | 86 b | 79 a |
| 25 | 80 a | 79 a | 78 a | 83 a |
| 30 | 69 b | 68 b | 71 b | 68 b |
| 35 | 48 c | 51 c | 55 c | 53 c |
| Germ tube branching (%) | | | | |
| 15 | 51 d | 41 d | 39 e | 44 d |
| 20 | 78 b | 70 b | 70 b | 72 b |
| 25 | 91 a | 89 a | 88 a | 94 a |
| 30 | 68 c | 71 b | 66 c | 77 b |
| 35 | 44 d | 51 c | 52 d | 49 d |
| Length of germ tubes (µm) | | | | |
| 15 | 391 e | 400 c | 381 e | 419 c |
| 20 | 492 b | 580 b | 515 b | 600 b |
| 25 | 894 a | 900 a | 1000 a | 991 a |
| 30 | 1009 a | 1001 a | 1100 a | 998 a |
| 35 | 600 b | 594 b | 608 b | 596 b |

Nota : - For each trait and within each column, values not followed by same letter are significantly different at $P = 0.05$ (Duncan's multiple range test).
– Data were obtained from a total of about 400 spores counted after 24 hours of incubation.

Table 6 – Variance analysis for the effect of temperature and maize cultivars (at 100 % R.H.) on spore germination and germ tube growth of *Curvularia pallescens*.

Tableau 6 – Analyses de variance pour l'effet de la température et de différents cultivars de maïs (à 100 % R.H.) sur la germination des spores et sur la croissance des tubes germinatifs de *Curvularia pallescens*.

| Source of variation | Degrees of freedom | Mean squares | | | |
|---|---|---|---|---|---|
| | | Germination | Intercalary germination | Germ tube branching | Length of germ tube |
| Cultivars | 3 | 12.99 | 30.85 | 19.48 | 475.52 |
| Error (a) | 16 | 6.10 | 5.93 | 9.91 | 2 121.10 |
| Temperature | 4 | 95.42 ** | 565.18 ** | 348.40 * | 103 158.10 ** |
| Cult. x Temp. | 12 | 65.20 ** | 66.87 ** | 49.91 ** | 20 314.39 ** |
| Error (b) | 80 | 10.14 | 21.16 | 16.10 | 66 100.42 |

* = significant at P=0.05

■ = significant at P=0.01

Table 7 – Comparison of spore germination of *Curvularia pallescens* on glass slides and maize leaves surfaces.

Tableau 7 – Comparaison de la germination des spores de *Curvularia pallescens* sur lames de verre et feuilles de maïs.

| Temperature °C | Relative Humidity % | Germination (%) | |
|---|---|---|---|
| | | Glass slide | Maize leaves |
| 25 | 95 | 84 c | 90 ■ |
| 25 | 100 | 89 bc | 98 a |
| 30 | 95 | 87 c | 97 ■ |
| 30 | 100 | ■ c | 93 b |

Nota : – Mean values in the same column not followed by the same letter are significantly different at P = 0.05 (Duncan's multiple range test).

## DISCUSSION

In this study the optimum temperature for germination was between 25 and 30°C. This is similar to the results of COCHRANE (1958) who found that optimum temperature for germination and growth of many non-pathogenic fungi was 25-30°C. Branching and germ tube elongation also had optimum temperature range of 25-30°C.

It has been recognized that most fungal spores must absorb water and swell before germination can occur (BONNER, 1948), it follows that water would be vital in the germination of fungal spores. Therefore it is possible that good germination and profuse germ tube growth of *Curvularia pallescens* spores at high RH (95 and 100 %) was due to the availability of enough moisture for imbibition and swelling prior to spore germination.

Based on the temperature - humidity interaction demonstrated in this study, it is inferred that neither temperature nor RH could be treated in isolation in studies of fungal spores involving germination of germ tube growth. GROOM & PANISSET (1933) noted that the optimum temperature range for *Penicillium chrysogenum* became narrower at lower RH. Also, BONNER (1948) found that RH for optimum germination of *Aspergillus niger* spores increased with increase in temperature. These results were also confirmed in this study where optimum temperature range for spore germination and germ tube growth was narrower at 95 % (25-30°C) than at 100 % (20-30°C). Thus a higher humidity may be needed to compensate for ■ higher temperature effect on biochemical activities prior to spore germination. It might be true that the temperature optima were governed by hydrolytic enzymes required to drive an enzymatic reaction to ■ stage which could in turn trigger the germination processes.

Germination of *Curvularia pallescens* spores on glass slides was significantly lower than that on leaf surfaces. Also, the maize cultivar from which the leaves

were obtained did not affect spore germination. It had been reported by KOSUGE & HEWITT (1964), that glucose and fructose which accumulate in water placed on grape leaves are sufficient to stimulate germination of spores as well as growth of *Botrytis cinerea*. A similar phenomenon may contribute to the greater spore germination and germ tube growth of *C. pallescens* on maize leaf surfaces than on glass slides. This opens up an area which deserves more study in order to know the type and amount of the compounds that affect spore germination on maize leaf surfaces.

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